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Research Article

BIOREMEDIATION OF YAMUNA WATER BY MONO AND DUAL BACTERIAL ISOLATES

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ABSTRACT
The aim of this study is to determine the potential use of microorganisms which are isolated and identified as *Rhodopseudomonas palustris*, *Rhodobacter spheroids*, *Bacillus subtilis*, *B.fusiformis*, *B.thurigiensis*, *B.cereus.*, *Lactobacillus* sp. and *E.coli* from Yamuna waste water. Among 31 bacterial isolates, only 8 bacterial isolates showed highest increment in highest reduction in BOD & COD in the selected water samples. Bacterial isolate 3 showed 79.49 and 85.46 percent reduction in BOD and COD and in combination with other isolates as using B_3B_4, BOD (77%) and COD (94%) and using B_1B_3, BOD and COD (63.23%) reduction was observed. Bacterial isolate 3 which was identified as *Bacillus subtilis* was proved to quite potent strain to remediate Yamuna waste water. Similarly other combinations as B_4B_5, B_2B_8, B_6B_8 and B_7B_8 also have shown effective reduction in BOD and COD levels rather than individual treatment.

Key Words: Bioremediation, Yamuna Water, Culture Combinations and Bacterial Isolates

INTRODUCTION
Untreated sewage on discharge to watercourse consumes oxygen, thus killing plant and animal life, and it would also cause a nuisance, as well as being a hazard to human health. The conventional biological treatment of sewage utilizes activated sludge, which is a random combination of microorganisms. Formulated microbial consortia represent a broad selection of microbial population. This composition degrades organic matter present in a wide range of substrates in a reproducible manner and also reduces the time required to carry out the same. The technology of Effective Microorganisms (EM) was developed during the 1970's at the University of Ryukyus, Okinawa, Japan (Sangakkara, 2002). EM was developed to increase the crop yield by enhancing the soil activity (Higa et al., 1994). But later, it has its application in wastewater treatment (Okuda et al., 1995). The EM has its wide application in the field of agriculture, natural farming, livestock, gardening, composting (Daly et al., 2005), bioremediation (Miyajjma et al., 1995) algal control and prawn culture. The EM suppresses soil borne pathogen and pest, promotes plant growth, improves soil fertility and yield of crops and used as feed additive for livestock. The EM treated sludge is used as fertilizer and the EM treated waste water is used in crop production as it is enriched with beneficial microorganisms (Higa et al., 1996).
Present research work is aimed to develop formulated microbial combinations for reducing the BOD load as well as COD level of Yamuna water sample. Different microbial combinations were formulated randomly primarily on the basis of their morphology, color, size, shape etc. A microbial combination having specific bacterial isolates was found to be capable of reducing the COD and BOD of Yamuna water up to specified permissible limits.

MATERIALS AND METHODS
Sampling and Isolation of bacterial strains
Yamuna water samples collected from four different sites: Yamuna water opposite Taj, Poiyaghat, Kailash Temple and Balkeshwar Temple. The bacterial isolates were isolated by serial dilution agar plate technique (Aneja, 2001). For this, the suspension of each sample was prepared by vortexing 1.0 ml of water sample in 9 ml sterilized distilled water and so prepared suspension of this dilution was aseptically spread on Petri plate having nutrient agar medium. The Petri plates were incubated at 37°C± 2°C for 48 hours. The bacterial colonies were isolated by streaking them on fresh plates containing nutrient agar medium. To avoid repeated isolation of same bacterial isolates, the colonies showing similar cultural characteristics, were obtained by repeated streaking of diluted suspension prepared from isolated bacterial colonies. The purified bacterial isolates were preserved on nutrient agar slants at 4˚C. All bacterial isolates were sub cultured after a time interval of 30 days.
Identification
The promising bacterial isolates were identified on the basis of their morphological, physiological and biochemical characteristic features (Claus & Berkley, 1986).

Waste water treatment

The waste water sample (5L) taken in a 10 L container was inoculated with 5% bacterial culture. After vigorous shaking it was divided into 12 portions of 250 ml each in 500 ml flasks and rest of the samples were divided into two portions of 1L each in 2L flasks (Prasad et al., 2010). All the cultures were incubated at 37° C. The standard Effective Microorganisms solution was collected from Microbiology Lab (Department of Botany), Dayalbagh Educational Institute, Agra. The extended solution of Effective Microorganisms were prepared by adding 5% stock solution of individual Effective Microorganisms as B_1: *Lactobacillus* sp., B_2: *Rhodobacter spheroids,* B_3: *Bacillus subtilis,* B_4: *B. fusiformis,* B_5: *Rhodopseudomonas palustris* ,B_6 : *B. thurigiensis.*, B_7 :*B. cereus* and *E.coli* as bacterial isolate 8 in Jaggary Solution. Further combinations were prepared in dual mode by inoculating 5 ml of each bacterial suspension in 1 L sample in following manner: B_1B_2, B_1B_3,B_1B_4,B_1B_5,B_1B_6, B_1B_7,B_1B_8, Similarly other combinations were prepared.

BOD and COD Estimation

BOD and COD values of untreated water sample and treated samples were determined by tritimetric method (APHA, 1998 & Aneja, 2001).

RESULTS AND DISCUSSION

In the present study, 31 bacterial isolates were isolated and cultured individually on Nutrient Agar Medium. Eight isolates with sharp clear zones were identified as *Lactobacillus* sp., *Rhodobacter spheroids, Bacillus subtilis, B. fusiformis, Rhodopseudomonas palustris, B. thurigiensis., B. cereus* and *E.coli.*

Untreated waste water samples of Yamuna River were treated with individual organisms and their dual combinations (consortia). Waste water samples showed an average pH of 7.3, average BOD value of 6.2 mg/L and COD of 54.4mg/L. After treating with individual bacteria and their mixtures the BOD and COD value of waste water sample were reduced based on degrading capacity of the organism. Maximum reduction in BOD (93.55%) and COD (73.9%) values were shown by *B.subtilis* followed by *Rhodopseudomonas palustris* reduced BOD to 74.2% and COD to 65.8% but minimum reduction was observed by *B.cereus* which reduced BOD (41.9% reduction) and COD (51.6% reduction) significantly (Table-1;Figure-1). Prasad et al., (2010) observed that mixture of all the organisms (*B.subtilis,B. licheniformis,B.amyloliuefaciens,Pseudomonas aeruginosa, Staphylococcus aureus* and *S.marsescens)* showed maximum activity with very low BOD value compared to individual treatment of lipid rich waste water.

Table 1: Percentage reduction in BOD and COD of Yamuna wastewater sample using Bacterial monocultures

S. No.	Bacterial Monocultures	OD(mg/L)	% reduction	COD(mg/L)	% reduction
B_1	*Lactobacillus*	4.0	35.49	25.3	53.5
B_2	*Rhodobacter spheroids*	2.4	61.29	21.3	60.85
B_3	*Bacillus subtilis*	0.4	93.55	14.2	73.90
B_4	*B. fusiformis*	2.1	66.13	19.3	64.53
B_5	*Rhodopseudomonas palustris*	1.6	74.20	18.6	65.81
B_6	*B. thurigiensis*	2.4	61.29	19.3	64.53
B_7	*B.cereus*	3.6	41.94	26.3	51.66
B_8	*E.coli*	2.6	58.07	20.3	62.69

Initial DO: 17.9 mg/L *Initial BOD: 6.2 mg/L* *Initial COD: 54.4 mg/L*

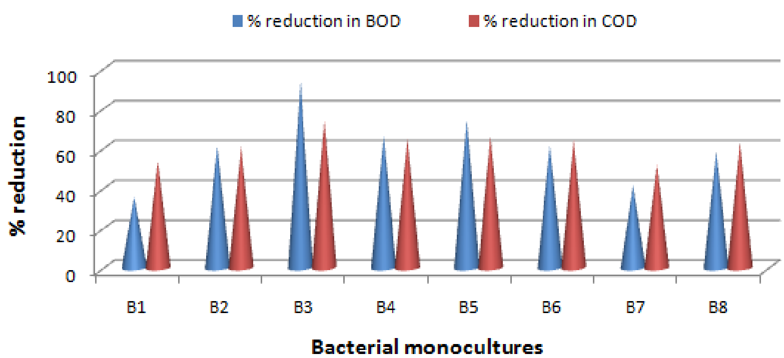

Figure 1: Determination of percent reduction in BOD and COD using bacterial monocultures

Table 2: Percentage reduction of DO, BOD & COD of Yamuna water sample using different bacterial combination

Bacterial isolates	BOD (mg/L)	%reduction/ %increment	COD (mg/L)	%reduction/ %increment
B_1+B_2	4.1	34	27	50.36
B_1+B_3	2.8	65	20	63.23
B_1+B_4	7.5	27	29	47
B_1+B_5	11.7	52	48	12
B_1+B_6	4.3	33	40	8
B_1+B_7	6.9	11	48	12
B_1+B_8	4.6	33	24	56
B_2+B_3	5.8	6.4	11.2	81
B_2+B_4	6.5	4.6	43.84	18.8
B_2+B_5	0.2	96.7	49.12	10.56
B_2+B_6	2.3	62.9	41.6	25.6
B_2+B_7	1.7	72.58	38.4	32
B_2+B_8	1.1	82.25	27.2	54.4
B_3+B_4	1.4	77.41	3.2	94.11
B_3+B_5	5.0	19.35	8.0	85.29
B_3+B_6	4.5	27.42	38.4	29.41
B_3+B_7	7.3	15	62	12
B_3+B_8	3.9	37.09	28.8	47.05
B_4+B_5	0.5	91.9	17.6	67.64
B_4+B_6	6.5	4.6	14.4	73.53
B_4+B_7	4.9	20.9	14.4	73.53
B_4+B_8	4.0	35.4	22.4	58.82
B_5+B_6	2.6	58.06	16	70.5
B_5+B_7	2.9	53.22	16	70.5
B_5+B_8	3.6	41.9	4.0	92.64
B_6+B_7	2.5	59.67	8.0	85.29
B_6+B_8	0.6	90.3	24	55.88
B_7+B_8	1.3	79.03	8.0	85.29

Initial DO: 17.9 mg/L *Initial BOD: 6.2 mg/L* *Initial COD: 54.4 mg/L*

Bacterial combinations were selected on the basis of their reduction in Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) of Yamuna water sample. Maximum reduction in BOD & COD using bacterial combination B_4B_5 was BOD (91.9%) and COD (67.6%) followed by combination $B_6B_{8,}$ BOD (65%) and COD (55.88%), using $B_3B_{4,}$ BOD (77%) and COD (94%), using $B_1B_{3,}$ BOD and COD (63.23%), using $B_2B_{8\ ,}$BOD (65%) and COD (54.4%), using $B_7B_{8,}$ BOD (79.03%) and COD (54.4%), using $B_7B_{8,}$ BOD (79.03%) and COD (85.29%)

respectively (Table-2). According to Pragati et al., (2008) maximum reduction in DO, BOD & COD were observed by bacterial combination B_4B_6 (48.38%), B_2B_3 (72.50%) & B_1B_3 (80.80%) whereas the minimum reduction by bacterial combination B_1B_2 (9.6%), B_5B_6 (50.0%) & B_4B_6 (73.2%) respectively.

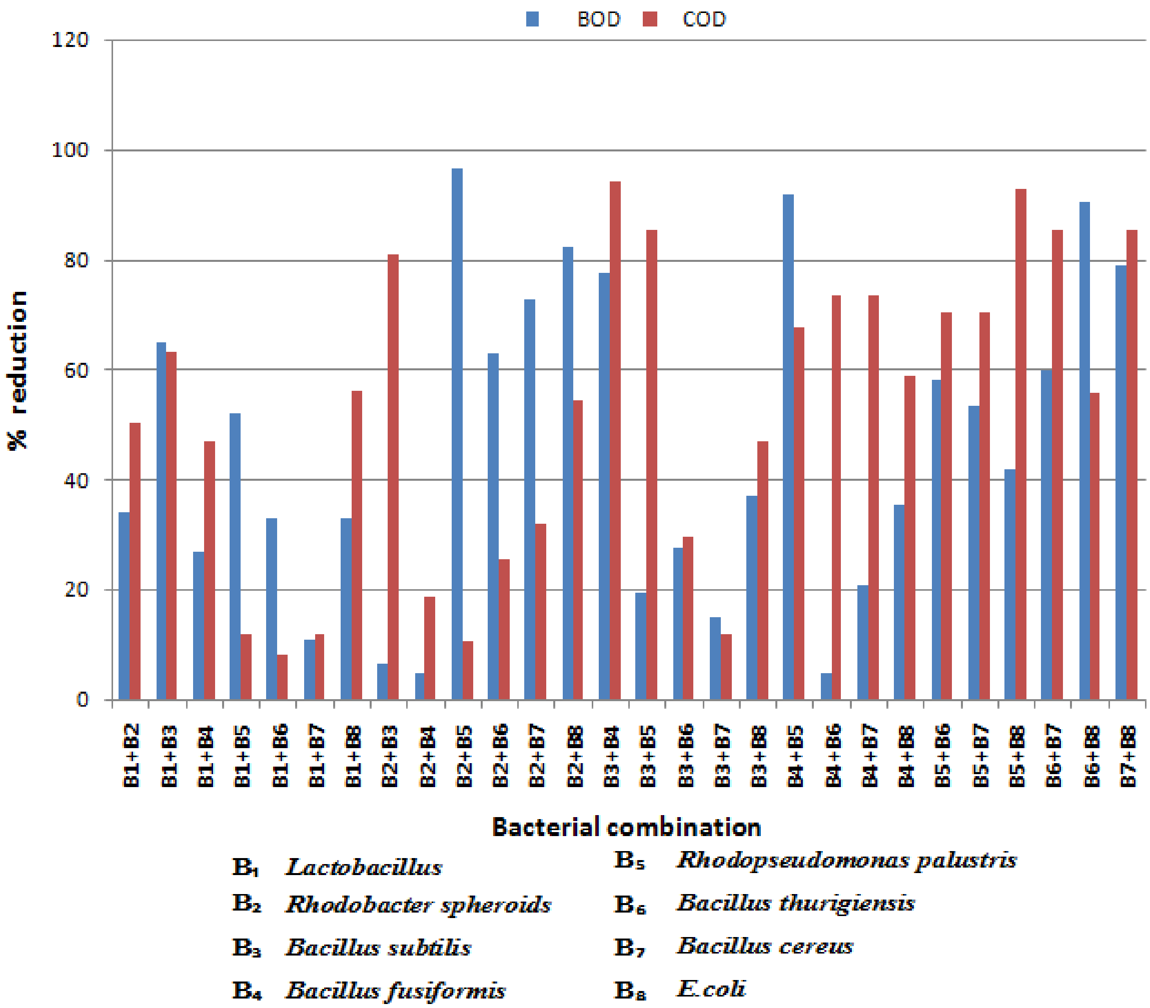

Figure 2: Determination of percent reduction in BOD and COD using bacterial combinations

Musa et al., (2010) reported that waste water discharged from the pineapple industry contributes to high levels of BOD, COD and Suspended Solids (SS). The high levels of COD concentrations in waste water are toxic to biological life and will affect aquatic environment. The bacterial pellet D, G and I showed a maximal COD reduction of 87%, 77% and 94% respectively after 3 days exposure to waste water. The waste water treatment using bacterial pellet showed COD reduction as compared to treatment using whole bacterial culture.

CONCLUSION

In this study different bacterial combinations were formulated using bacterial monocultures (1,2,3,4,5,6,7,8) for waste water treatment of Yamuna river. The role of *B.subtilis* in reduction of BOD and COD levels is high compared to other bacteria, similarly its combinations with *Lactobacillus* sp. and *B.fusiformis* showed remarkable reduction efficiency in BOD and COD. The average BOD value was reduced from 6.2mg/L to 0.4 mg/L and COD level was reduced from 54.4 mg/L to 14.2 mg/L.

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